

CLAIMS

I claim:

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1. A method of reducing formation of artifacts in a nucleic acid amplification reaction, the method comprising
using a template-deficient oligonucleotide as at least one of the oligonucleotides in the nucleic acid amplification reaction,
wherein the template-deficient oligonucleotide comprises one or more template-deficient nucleotides,
wherein the number and composition of template-capable nucleotides 3' of the template-deficient nucleotide closest to the 3' end of the template-deficient oligonucleotide is sufficient to allow the template-capable nucleotides 3' of the template-deficient nucleotide closest to the 3' end alone to effectively prime nucleic acid synthesis in the nucleic acid amplification reaction.
 2. The method of claim 1 wherein the one or more template-deficient nucleotides are at or near the 5' end of the template-deficient oligonucleotide.
 3. The method of claim 1 wherein the template-deficient oligonucleotide comprises two or more template-deficient nucleotides, wherein at least two of the two or more template-deficient nucleotides are adjacent.
 4. The method of claim 3 wherein the two or more adjacent template-deficient nucleotides are within three nucleotides of the 5' end of the template-deficient oligonucleotide.
 5. The method oligonucleotide of claim 1 wherein the template-deficient nucleotides are selected from the group consisting of modified nucleotides, derivatized nucleotides, ribonucleotides, and nucleotide analogs.
 6. The method oligonucleotide of claim 1 wherein the template-deficient oligonucleotide comprises two or more template-deficient nucleotides, wherein at least two of the two or more template-deficient nucleotides are different.
 7. The method of claim 1 wherein the template-deficient oligonucleotide comprises two or more template-deficient nucleotides, wherein at least two of the
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two or more template-deficient nucleotides are template-deficient for different reasons.

8. The method of claim 5 wherein the template-deficient nucleotides are modified nucleotides.

9. The method of claim 5 wherein the modified nucleotides are abasic nucleotides.

10. The method of claim 5 wherein the template-deficient nucleotides are selected from the group consisting of abasic nucleotides, nucleotides with an inverted base, fluoro substituted nucleotides, alkyl substituted nucleotides, nucleotides with phenyl substituted ethers, nucleotides with substituted thioethers, nucleotides with phosphate esters, α -nucleotides, 2',3'-dideoxy nucleotides, ribonucleotides, nucleotides derivatized with biotin, nucleotides derivatized with amine, nucleotides derivatized with Hex, nucleotides derivatized with Tet, nucleotides derivatized with Fam, nucleotides derivatized with fluorescein, nucleotides derivatized with rhodamine, nucleotides derivatized with alkaline phosphatase, nucleotides derivatized with horseradish peroxidase, nucleotides derivatized with spacers, nucleotides derivatized with cholesteryl, nucleotides derivatized with DNP-TEG, nucleotides derivatized with psoralen cross-linkers, nucleotides derivatized with intercalating agents, and nucleotides derivatized with PNA conjugates.

11. The method of claim 1 wherein the nucleic acid amplification reaction does not involve cycle sequencing.

12. The method of claim 11 wherein the nucleic acid amplification reaction does not require linear amplification via thermal cycling.

13. The method of claim 12 wherein the nucleic acid amplification reaction does not involve linear amplification via thermal cycling.

14. The method of claim 1 wherein the nucleic acid amplification reaction involves exponential amplification via thermal cycling.

15. The method of claim 14 wherein the nucleic acid amplification reaction requires exponential amplification via thermal cycling.

16. The method of 14 wherein the nucleic acid amplification reaction involves the polymerase chain reaction.

17. The method of claim 1 wherein the nucleic acid amplification does not involve thermal cycling.

18. The method of 17 wherein the nucleic acid amplification is rolling circle amplification.

Sub 19. The method of claim 1 wherein the nucleic acid amplification reaction is selected from the group consisting of exponential rolling circle amplification (ERCA), and rolling circle amplification (RCA), multiple displacement amplification (MDA), strand displacement amplification (SDA), nucleic acid sequence based amplification (NASBA), transcription-mediated amplification (TMA), polymerase chain reaction (PCR), self-sustained sequence replication (3SR), amplification with Q β replicase, and cycle sequencing.

20. The method of claim 1 wherein the template-deficient oligonucleotide is a primer.

21. The method of claim 20 wherein all of the primers used in the nucleic acid amplification reaction are template-deficient.

22. The method of claim 1 wherein all of the oligonucleotides used in the nucleic acid amplification reaction are template-deficient.

Sub 23. A method of reducing formation of artifacts in a nucleic acid amplification reaction, the method comprising

using a template-deficient oligonucleotide as at least one of the oligonucleotides in the nucleic acid amplification reaction,

wherein the nucleic acid amplification reaction does not involve cycle sequencing.

24. The method of claim 23 wherein the nucleic acid amplification reaction does not require linear amplification via thermal cycling.

25. The method of claim 24 wherein the nucleic acid amplification reaction does not involve linear amplification via thermal cycling.

26. The method of claim 23 wherein the nucleic acid amplification does not involve thermal cycling.

Sub 27. The method of claim 26 wherein the nucleic acid amplification is rolling circle amplification.

28. The method of claim 23 wherein the nucleic acid amplification reaction involves exponential amplification via thermal cycling.

29. The method of claim 28 wherein the nucleic acid amplification reaction requires exponential amplification via thermal cycling.

30. The method of claim 28 wherein the nucleic acid amplification reaction involves the polymerase chain reaction.

Sub 31. The method of claim 23 wherein the nucleic acid amplification reaction is selected from the group consisting of exponential rolling circle amplification (ERCA), and rolling circle amplification (RCA), multiple displacement amplification (MDA), strand displacement amplification (SDA), nucleic acid sequence based amplification (NASBA), transcription-mediated amplification (TMA), polymerase chain reaction (PCR), self-sustained sequence replication (3SR), and amplification with Q β replicase.

32. The method of claim 23 wherein the template-deficient oligonucleotide comprises one or more template-deficient nucleotides.

Sub 33. The method of claim 32 wherein the one or more template-deficient nucleotides are at or near the 5' end of the template-deficient oligonucleotide.

34. The method of claim 32 wherein the template-deficient oligonucleotide comprises two or more template-deficient nucleotides, wherein at least two of the two or more template-deficient nucleotides are adjacent.

35. The method of claim 34 wherein the two or more adjacent template-deficient nucleotides are within three nucleotides of the 5' end of the template-deficient oligonucleotide.

36. The method oligonucleotide of claim 32 wherein the template-deficient nucleotides are selected from the group consisting of modified nucleotides, derivatized nucleotides, ribonucleotides, and nucleotide analogs.

37. The method oligonucleotide of claim 32 wherein the template-deficient oligonucleotide comprises two or more template-deficient nucleotides, wherein at least two of the two or more template-deficient nucleotides are different.

38. The method of claim 32 wherein the template-deficient oligonucleotide comprises two or more template-deficient nucleotides, wherein at least two of the two or more template-deficient nucleotides are template-deficient for different reasons.

39. The method of claim 36 wherein the template-deficient nucleotides are modified nucleotides.

40. The method of claim 36 wherein the modified nucleotides are abasic nucleotides.

41. The method of claim 36 wherein the template-deficient nucleotides are selected from the group consisting of abasic nucleotides, nucleotides with an inverted base, fluoro substituted nucleotides, alkyl substituted nucleotides, nucleotides with phenyl substituted ethers, nucleotides with substituted thioethers, nucleotides with phosphate esters, α -nucleotides, 2',3'-dideoxy nucleotides, ribonucleotides, nucleotides derivatized with biotin, nucleotides derivatized with amine, nucleotides derivatized with Hex, nucleotides derivatized with Tet, nucleotides derivatized with Fam, nucleotides derivatized with fluorescein, nucleotides derivatized with rhodamine, nucleotides derivatized with alkaline phosphatase, nucleotides derivatized with horseradish peroxidase, nucleotides derivatized with spacers, nucleotides derivatized with cholesteryl, nucleotides derivatized with DNP-TEG, nucleotides derivatized with psoralen cross-linkers, nucleotides derivatized with intercalating agents, and nucleotides derivatized with PNA conjugates.

42. The method of claim 32 wherein the number and composition of template-capable nucleotides 3' of the template-deficient nucleotide closest to the 3' end is sufficient to allow the template-capable nucleotides 3' of the template-deficient nucleotide closest to the 3' end alone to effectively prime nucleic acid synthesis in the nucleic acid amplification reaction.

43. The method of claim 23 wherein the template-deficient oligonucleotide is a primer.

44. The method of claim 43 wherein all of the primers used in the nucleic acid amplification reaction are template-deficient.

45. The method of claim 23 wherein all of the oligonucleotides used in the nucleic acid amplification reaction are template-deficient.

~~46. A method of reducing formation of artifacts in a nucleic acid amplification reaction, the method comprising~~

~~using a template-deficient oligonucleotide as at least one of the oligonucleotides in the nucleic acid amplification reaction,~~

~~wherein the nucleic acid amplification reaction involves exponential amplification via thermal cycling.~~

~~47. The method of claim 46 wherein the nucleic acid amplification reaction requires exponential amplification via thermal cycling.~~

~~48. The method of 46 wherein the nucleic acid amplification reaction involves the polymerase chain reaction.~~

~~49. The method of claim 46 wherein the template-deficient oligonucleotide comprises one or more template-deficient nucleotides.~~

~~50. A template-deficient oligonucleotide comprising one or more template-deficient nucleotides,~~

~~wherein the number and composition of template-capable nucleotides 3' of the template-deficient nucleotide closest to the 3' end is sufficient to allow the template-capable nucleotides 3' of the template-deficient nucleotide closest to the 3' end alone to effectively prime nucleic acid synthesis in the nucleic acid amplification reaction.~~

51. The template-deficient oligonucleotide of claim 50 wherein the one or more template-deficient nucleotides are at or near the 5' end of the template-deficient oligonucleotide.

52. The template-deficient oligonucleotide of claim 50 wherein the template-deficient oligonucleotide comprises two or more template-deficient nucleotides, wherein at least two of the two or more template-deficient nucleotides are adjacent.

53. The template-deficient oligonucleotide of claim 52 wherein the two or more adjacent template-deficient nucleotides are within three nucleotides of the 5' end of the template-deficient oligonucleotide.

54. The template-deficient oligonucleotide of claim 50 wherein the template-deficient nucleotides are selected from the group consisting of modified nucleotides, derivatized nucleotides, ribonucleotides, and nucleotide analogs.

55. The template-deficient oligonucleotide of claim 50 wherein the template-deficient oligonucleotide comprises two or more template-deficient nucleotides, wherein at least two of the two or more template-deficient nucleotides are different.

56. The template-deficient oligonucleotide of claim 50 wherein the template-deficient oligonucleotide comprises two or more template-deficient nucleotides, wherein at least two of the two or more template-deficient nucleotides are template-deficient for different reasons.

57. The template-deficient oligonucleotide of claim 54 wherein the template-deficient nucleotides are modified nucleotides.

58. The template-deficient oligonucleotide of claim 54 wherein the modified nucleotides are abasic nucleotides.

59. The template-deficient oligonucleotide of claim 54 wherein the template-deficient nucleotides are selected from the group consisting of abasic nucleotides, nucleotides with an inverted base, fluoro substituted nucleotides, alkyl substituted nucleotides, nucleotides with phenyl substituted ethers, nucleotides with substituted thioethers, nucleotides with phosphate esters, α -nucleotides, 2',3'-dideoxy nucleotides, ribonucleotides, nucleotides derivatized with biotin, nucleotides derivatized with amine, nucleotides derivatized with Hex, nucleotides derivatized with Tet, nucleotides derivatized with Fam, nucleotides derivatized with fluorescein, nucleotides derivatized with rhodamine, nucleotides derivatized with alkaline phosphatase, nucleotides derivatized with horseradish peroxidase, nucleotides

derivatized with spacers, nucleotides derivatized with cholesteryl, nucleotides derivatized with DNP-TEG, nucleotides derivatized with psoralen cross-linkers, nucleotides derivatized with intercalating agents, and nucleotides derivatized with PNA conjugates.

60. A kit for nucleic acid amplification, the kit comprising a template-deficient primer, wherein the template-deficient primer comprises one or more template-deficient nucleotides,

wherein the number and composition of template-capable nucleotides 3' of the template-deficient nucleotide closest to the 3' end is sufficient to allow the template-capable nucleotides 3' of the template-deficient nucleotide closest to the 3' end alone to effectively prime nucleic acid synthesis in the nucleic acid amplification reaction.

61. The kit of claim 60 wherein the one or more template-deficient nucleotides are at or near the 5' end of the template-deficient primer.

62. The kit of claim 60 wherein the template-deficient primer comprises two or more template-deficient nucleotides, wherein at least two of the two or more template-deficient nucleotides are adjacent.

63. The kit of claim 62 wherein the two or more adjacent template-deficient nucleotides are within three nucleotides of the 5' end of the template-deficient primer.

64. The kit of claim 60 wherein the template-deficient nucleotides are selected from the group consisting of modified nucleotides, derivatized nucleotides, ribonucleotides, and nucleotide analogs.

65. The kit of claim 60 wherein the template-deficient primer comprises two or more template-deficient nucleotides, wherein at least two of the two or more template-deficient nucleotides are different.

66. The kit of claim 60 wherein the template-deficient primer comprises two or more template-deficient nucleotides, wherein at least two of the two or more template-deficient nucleotides are template-deficient for different reasons.

67. The kit of claim 64 wherein the template-deficient nucleotides are modified nucleotides.

68. The kit of claim 64 wherein the modified nucleotides are abasic nucleotides.

69. The kit of claim 64 wherein the template-deficient nucleotides are selected from the group consisting of abasic nucleotides, nucleotides with an inverted base, fluoro substituted nucleotides, alkyl substituted nucleotides, nucleotides with phenyl substituted ethers, nucleotides with substituted thioethers, nucleotides with phosphate esters, α -nucleotides, 2',3'-dideoxy nucleotides, ribonucleotides, nucleotides derivatized with biotin, nucleotides derivatized with amine, nucleotides derivatized with Hex, nucleotides derivatized with Tet, nucleotides derivatized with Fam, nucleotides derivatized with fluorescein, nucleotides derivatized with rhodamine, nucleotides derivatized with alkaline phosphatase, nucleotides derivatized with horseradish peroxidase, nucleotides derivatized with spacers, nucleotides derivatized with cholesteryl, nucleotides derivatized with DNP-TEG, nucleotides derivatized with psoralen cross-linkers, nucleotides derivatized with intercalating agents, and nucleotides derivatized with PNA conjugates.

70. The kit of claim 60 wherein the nucleic acid amplification reaction does not involve cycle sequencing.

71. The kit of claim 70 wherein the nucleic acid amplification reaction does not require linear amplification via thermal cycling.

72. The kit of claim 74 wherein the nucleic acid amplification reaction does not involve linear amplification via thermal cycling.

73. The kit of claim 63 wherein the nucleic acid amplification reaction involves exponential amplification via thermal cycling.

74. The kit of claim 76 wherein the nucleic acid amplification reaction requires exponential amplification via thermal cycling.

75. The kit of claim 76 wherein the nucleic acid amplification reaction involves the polymerase chain reaction.

76. The kit of claim 63 wherein the nucleic acid amplification reaction is selected from the group consisting of exponential rolling circle amplification (ERCA), and rolling circle amplification (RCA), multiple displacement amplification (MDA), strand displacement amplification (SDA), nucleic acid sequence based amplification (NASBA), transcription-mediated amplification (TMA), polymerase chain reaction (PCR), self-sustained sequence replication (3SR), and amplification with Q β replicase.

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